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CONCERNING THE PRESENCE OF DIASTASE IN CERTAIN RED ALGAE

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Introduction

In most starch-forming plants the starch grains are deposited in the plastids. The work of both early and recent investigators shows, however, that this is probably never true for the red algae. In this group of plants the grains are described as being formed in the cytoplasm, outside the plastids and often apparently quite independent of them.

In the red algae the grains, which resemble the starch grains of higher plants, do not usually give the customary blue color when treated with iodine or zinc chloriodide. In a few species, for example *Lorencia* sp., *Polysiphonia nigrescens*, and *Ceramium tenuissimum*,[1] the color with iodine varies from violet to almost blue, but in most species it ranges from light brown to wine red. There is such a wide divergence of reaction in the different forms tested that OLTMANNS[2] suggests the possibility that each species will be found to give its own characteristic color reaction.

SAIKI[3] found that by applying strong solutions of malt extract to commercial food preparations made from various red algae, such as *Gelidium* sp., *Porphyra* sp., and *Chondrus crispus*, no digestion whatever of the carbohydrates took place. From this and other experiments he concludes that the polysaccharid carbohydrates in these algal food preparations are not readily transformed into sugar by carbohydrate-digesting enzymes of animal origin, and scarcely more so by vegetable enzymes or bacteria.

KÖNIG and BETTELS[4] hydrolyzed, with acids, the carbohydrates in *Porphyra* sp., *Gelidium* sp., and commercial compounds composed of red algae. Their results showed the presence of galactose, fructose, and glucose in varying quantities. In carrageen (*Chondrus crispus*), MÜTHER and TOLLENS[5] found galactose and probably other hexoses belonging to the fructose and glucose groups.

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[1] OLTMANNS, F., Morphologie und Biologie der Algen **2**:148.

[2] *Ibid.*

[3] SAIKI, T., The digestibility and utilization of some polysaccharid carbohydrates derived from the lichens and red algae. Jour. Biol. Chem. **2**:251–265. 1906.

[4] KÖNIG, J., and BETTELS, J., Die Kohlenhydrate der Meeresalgen und daraus hergestellter Erzeugnisse. Zeitschr. Untersuch. Nahrungs- und Genussmittel **10**: 457. 1905.

The grains in the red algae often show the physical characteristics of the common starch grains of green plants as to hilum, striation, and effect of polarized light, but their place of deposition, their behavior toward iodine and zinc chloriodide, their sugar extracts, and their apparent resistance to the action of malt extract would tend to prove that they are not true starch. The grains appear and disappear at various times during the life of the plant, thus suggesting the presence of an enzyme which is an active agent in bringing about their decomposition.

The following experiments were performed that further evidence might be gained as to the nature of the substance composing the starchlike grains in the red algae, by determining whether or not the diastase that acts on the grains in these plants will also digest the common starch isolated from the higher plants. So far as is known to the writer, this is the first attempt in this direction, and a preliminary statement of the results is herewith recorded.

Material and methods

I. SPECIES FROM WHICH EXTRACTS WERE MADE

Polysiphonia variegata Ag., *Dasya elegans* Ag., *Agardhiella tenera* (J. Ag.) Schmitz, and *Ceramium*[6] lent themselves most readily to experimentation because of their abundance and the ease with which they could be obtained. *Grinnellia americana* Harv., *Griffiithsia globifera* J. Ag., and *Chondrus crispus* (L.) Stack. were also used and tended to show the same results as those obtained from the foregoing species, but sufficient quantities of the latter named plants could not be procured at the time of these experiments to warrant the drawing of definite conclusions.

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[5] MÜTHER, A., and TOLLENS, B., Ber. Deutsch. Chem. Gesells. 37:298–305, 306–311. 1904.

[6] Several species were used in the same mass of material.

2. METHOD OF EXTRACTION AND AMOUNTS OF FINAL PRECIPITATES

The extracts were made according to modified Buchner and Litner methods. Fresh vigorous algae were taken from the sea water and quickly rinsed under the tap. The excess tap water was removed by filter paper or cheese cloth. The algal material was then immersed in 95 per cent alcohol for 20 minutes. Having been freed from excess alcohol, it was next plunged into acetone for 10 minutes. The excess of this solution having been removed, the material was placed in fresh acetone for two minutes, partially dried by suction, and placed in an oven at 35°–40° C. until dry. Upon being taken from the oven, the dried material was cooled in a desiccator, thoroughly pulverized, placed in three times its amount of 20 per cent alcohol, and left standing 18–24 hours. At the end of this period the substance was filtered by suction through a Buchner funnel lined with cheese cloth and filter paper (it was necessary to filter the material by suction because of its viscosity), and to the liquid thus drawn off was added two and one-half times its volume of 95 per cent alcohol. After being filtered as above, the precipitate was washed in equal parts of ether and absolute alcohol, dried in a desiccator, and after being pulverized the extract was ready for use. The extraction treatment did not remove all of the color, so that the precipitates varied from brownish to light red.

Because of the difficulty experienced in filtering, the final precipitates were probably far from pure, but the following figures will show the amounts of precipitates obtained from the different species:

Polysiphonia variegata	21.00	grams powder*	—0.15	gram final ppt.
Ceramium	51.00	“ “	—0.15	“ “ “
Dasya elegans	53.03	“ “	—0.06	“ “ “
Agardhiella tenera	53.465	“ “	—0.23	“ “ “
Agardhiella tenera	17.00	“ “	—0.106	“ “ “

*Obtained after material had been dehydrated, desiccated, and pulverized.

3. ALGAL EXTRACTS AND COMMERCIAL DIASTASE

Solutions of various concentrations were made from the algal extracts, but the one giving the best results consisted of 0.03 gm. of extract dissolved in 5 cc. of distilled water. Owing to the nature of the extracts, solutions of this concentration were quite viscous. In most cases the viscosity would about equal that of a 75 per cent solution of glycerine and water. That a check might be had as to the time required for action and as to the final results, solutions of commercial diastase were also used. The solutions were of the same concentration as those of the algal extracts and were applied and tested in the same manner in every case. Both Eimer and Amend's and Merck's diastase were used, with no apparent difference in the results.

4. STARCH PASTE

Corn starch was used in all experiments. The paste used was made by adding 0.25 gm. of starch to 100 cc. of water and boiling 6 minutes. The boiling, though done slowly and in an Erlenmeyer flask plugged with cotton, probably caused 10 per cent of the water to be vaporized. No water was added to make up for this loss. New solutions of paste were made for each series of experiments.

5. CONTROL MEASURES

To determine as far as possible all sources of error, the following control measures were taken:

a) The "A, B, and C" parts of Fehling's solution were mixed just preceding each series of experiments.

b) Tubes of untreated paste were tested each time and in the same manner as those tubes containing the paste plus the extracts.

c) To detect any error due to the presence of an active agent in the distilled water, tubes of the paste were treated each time with distilled water only and tested in the regular manner.

d) Toluol was used as a sterilizing medium, except in the Van Tieghem tests, in which 10 per cent alcohol was used.

e) All containers and instruments were carefully sterilized with a strong solution of potassium bichromate and sulphuric acid.

6. TEST METHODS

A. *Digestion in test tubes*

The proportionate amounts of starch, algal extracts, commercial diastase, and distilled water used in each series of tests were as follows:

Test tube no. 1–5 cc. starch paste plus 1 cc. algal extract
“ “ “ 2–5 cc. “ “ “ 1 cc. “ “
“ “ “ 3–5 cc. “ “ “ 1 cc. commercial diastase
“ “ “ 4–5 cc. “ “ “ 2 cc. “ “
“ “ “ 5–5 cc. “ “ “ 1 cc. distilled water
“ “ “ 6–5 cc. “ “ “ 2 cc. “ “
“ “ “ 7–5 cc. untreated starch paste
“ “ “ 8–2 cc. algal extract solution
“ “ “ 9–2 cc. commercial diastase solution

When the experiments were to run for a longer period, the amounts were increased, but the same proportions were maintained. At the end of certain periods, indicated in the tables, 2 cc. of the solution were taken from tube number one and placed in two smaller tubes, 1 cc. in each tube. This process was repeated until test samples had been taken from each of the 7 tubes. One of each pair of small tubes was treated with iodine and the other with Fehling's solution. The experiments were all kept at room temperature, which was rather low, the daily average being 18°6 C.

B. *Digestion in Van Tieghem cells*

To determine microscopically the effect of the algal extract upon unboiled starch, small quantities of dry corn starch were placed in four Van Tieghem cells. Three of these were treated with extracts from *Polysiphonia*, *Agardhiella*, and *Ceramium*, the fourth with distilled water to act as a control.

7. TERMS USED IN TABLES TO DESIGNATE COLOR REACTIONS AND AMOUNTS OF PRECIPITATES

That the standard for the designation of iodine color reactions and for the amounts of copper precipitate might be as nearly as possible the same for each series of experiments, two sets of terms were adopted. These may be interpreted in the following manner by referring to PRANG'S *Standard of colors* (1898):

IODINE COLOR REACTIONS

As specified in tables	As illustrated by chart
1. Blue	Plate I, 1–B
2. Tinge of purple	“ I, 1–BV
3. Blue-purple	“ II, 1–BV
4. Purple	“ II, 1–V
5. Light purple	“ II, 2–V
6. Very light purple	“ II, 3–V
7. Purple-brown	“ V, 5–O
8. Brown	“ III, 3–OYO

*Fehling's solution precipitates**

As specified in tables	Algal extract	Commercial diastase
1. Trace	Plate II, 6–OYO	Plate II, 6–YYG
2. Very slight	" II, 5–O	" II, 4–Y
3. Slight	" I, 6–OYO	" II, 2–Y
4. Small	" I, 5–O	" I, 4–Y
5. Fair	" I, 4–ORO	" I, 3–Y
6. Medium	" I, 3–ORO	" I, 2–Y
7. Marked	" I, 3–RO	" I, 1–Y
8. Very marked	" I, 2–RO	" I, Y

*Although the precipitates from the two different extracts were of different colors, the terms used designate, as nearly as could be estimated, equal amounts of the two precipitates.

These designations were of course only relative, but they served very well as a guide. The colors designated in the tables were recorded within a few minutes after the application of the testing reagents.

Results

1. DIGESTION IN TEST TUBES

Tables I–IV were chosen promiscuously from a large number of tables, and are used as types to show the effect of the algal extract on starch paste and its rate of action in comparison with that of commercial diastase.

TABLE I

EXTRACT FROM CERAMIUM

Amounts of various solutions	After 11 hours		After 24 hours	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste	Tinge of purple	Trace	Blue-purple	Slight
2 cc. algal extract+5 cc. starch paste	Blue-purple	Slight	Purple	Small
1 cc. commercial diastase+5 cc. starch paste	Light purple	Fair	Light purple	Medium
2 cc. commercial diastase+5 cc. starch paste	Very light purple	Fair	Light brown	Marked
1 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
Control, 5 cc. starch paste	Blue	None	Blue	None
2 cc. algal extract	....	None	....	None
2 cc. commercial diastase	....	Medium	....	Medium

The action of the algal extract is very slow when compared with that of commercial diastase, as is shown in the preceding tables I–IV. To determine whether the action of the algal extract would

TABLE II

EXTRACTS FROM POLYSIPHONIA VARIEGATA

AMOUNTS OF VARIOUS SOLUTIONS	AFTER 12 HOURS		AFTER 36 HOURS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste............	Tinge of purple	Slight	Light purple	Medium
2 cc. algal extract+5 cc. starch paste............	Blue-purple	Small	Very light purple	Medium
1 cc. commercial diastase+5 cc. starch paste.........	Purple-brown	Marked	Brown	Very marked
2 cc. commercial diastase+5 cc. starch paste.........	Brown	Marked	Brown	Very marked
1 cc. distilled water+5 cc. starch paste............	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste............	Blue	None	Blue	None
Control, 5 cc. starch paste .	Blue	None	Blue	None
2 cc. algal extract.........	....	None	....	None
2 cc. commercial diastase...	....	Medium	....	Medium

TABLE III

EXTRACTS FROM AGARDHIELLA TENERA

AMOUNTS OF VARIOUS SOLUTIONS	AFTER 16 HOURS		AFTER 28 HOURS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste............	Blue	None	Tinge of purple	Trace
2 cc. algal extract+5 cc. starch paste............	Tinge of purple	Trace	Blue-purple	Slight
1 cc. commercial diastase+5 cc. starch paste	Purple-brown	Medium	Brown	Very marked
2 cc. commercial diastase+5 cc. starch paste.........	Brown	Medium	Brown	Very marked
1 cc. distilled water+5 cc. starch paste............	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste............	Blue	None	Blue	None
Control, 5 cc. starch paste .	Blue	None	Blue	None
2 cc. algal extract.........	....	None	....	None
2 cc. commercial diastase...	....	Medium	....	Medium

cease before digestion was complete, experiments were set up as before but allowed to run for a longer time. The results are shown in table V.

TABLE IV

EXTRACT FROM DASYA ELEGANS

AMOUNTS OF VARIOUS SOLUTIONS	AFTER 17 HOURS		AFTER 25 HOURS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
1 cc. algal extract+5 cc. starch paste	Blue	None	Blue ?	Trace
2 cc. algal extract+5 cc. starch paste	Blue ?	Trace	Tinge of purple	Very slight
1 cc. commerical diastase+5 cc. starch paste	Very light purple	Marked	Brown	Very marked
2 cc. commercial diastase+5 cc. starch paste	Purple-brown	Marked	Brown	Very marked
1 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
2 cc. distilled water+5 cc. starch paste	Blue	None	Blue	None
Control, 5 cc. starch paste	Blue	None	Blue	None
2 cc. algal extract	....	None	....	None
2 cc. commerical diastase	....	Medium	....	Medium

TABLE V

EFFECTS OF LONGER CONTINUED ACTION OF THE ALGAL EXTRACTS AND THEIR COMPARATIVE RATES OF ACTION

AMOUNTS OF SOLUTION ON 5 CC. OF STARCH PASTE	AFTER 3 DAYS		AFTER 6 DAYS	
	Iodine color reactions	Amts. ppt. with Fehling's solution	Iodine color reactions	Amts. ppt. with Fehling's solution
2 cc. Agardhiella extract	Purple	Fair	Very light purple	Very marked
3 cc. Agardhiella extract	Light purple	Medium	Very light purple	Very marked
2 cc. Ceramium* extract	Tinge of purple	Very slight	Blue-purple	Slight
3 cc. Ceramium* extract	Blue-purple	Slight	Purple	Fair
2 cc. Polysiphonia extract	Blue-purple	Medium	Very light purple	Very marked
3 cc. Polysiphonia extract	Purple	Marked	Very light purple	Very marked
2 cc. distilled water	Blue	None	Blue	None
3 cc. distilled water	Blue	None	Blue	None
Control, 10 cc. starch paste	Blue	None	Blue	None

*It took nine days for *Ceramium* to complete digestion.

2. DIGESTION IN VAN TIEGHEM CELLS

The cells were examined daily and it was found that in those containing the starch treated with the algal extracts, very noticeable corrosion of the grains was taking place. The rates of digestion agreed very well with the tabulated results in table V, *Polysiphonia* and *Agardhiella* acting more rapidly than *Ceramium.* The results thus obtained in the first trial were confirmed by repeating the experiment several times. Marked acceleration of corrosion was obtained by placing the cells in a warming oven at a temperature of 26° C. No corrosion could be detected in the control cells.

Discussion

That the disappearance of the starchlike grains in the living tissues of the red algae is due to the presence of some catalytic agent, which may be extracted and applied to corn starch and its derivatives with positive effects, is demonstrated by the results recorded in the foregoing tables. The action of this extract when applied to starch paste is very similar to that of commercial diastase. This is shown by the fact that in both cases the digestion of the starch appeared to be a series of processes leading more or less gradually from the amyloses down through the dextrins until amylolytic action was complete. This is brought out clearly not only by variations in color, but also by the fact that often when the iodine would indicate a marked degree of change in the solution, still the Fehling's test would show the presence of but very little sugar. It would appear from this that the algal extract, like the diastase of the higher plants, is not one simple enzyme but a complex of amylases and dextrinases. Then, if we may argue from analogy, the grains in the red algae, commonly referred to as starch, are probably, as MEYER[7] and others suggest, a combination of both starch and dextrins, or, as BÜTSCHLI[8] thinks, possibly a transitional stage between amyloporphyrin and amyloerythrin.

No direct evidence was obtained by experimentation as to whether or not this carbohydrate-digesting enzyme of the red algae might also act as a synthetic agent in forming the grains in question. BELZUNG[9] found that in some species the younger the grains the more nearly they came to giving the normal blue color reaction when treated with iodine. Hence, if this enzyme does act synthetically, it appears that, in some cases at least, the action is just the reverse of that which takes place in some of the higher plants, for example, in *Pisum sativum*, in which the erythrodextrins are formed first, the amylases not appearing until the grains are almost mature.

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[7] MEYER, A., Untersuchungen über die Stärkekörner. Jena. 1895.

[8] BÜTSCHLI, O., Notiz über d. sogen. Florideen Stärke. Verh. Naturf. Med. Ver. Heidelberg N.F. **7**:519–528. 1903.

The difference in rate of amylolytic action between the algal extract and the commercial diastase is very noticeable. This is already indicated in tables I–IV. This variation may have been due to difference in concentration. The algal extracts were known to be far from pure, and it was for this reason that solutions of rather high concentration were applied to the starch paste. Table V indicates that there is also considerable dissimilarity in digestive power between the algal extracts themselves. Here again is the possibility that this may be attributed to an inequality in purity, for the extracts did not all present the same degree of difficulty in filtration. However, the explanation is hardly to be looked for in this direction, for although *Agardhiella* and *Polysiphonia* extracts were more difficult to filter than was the *Ceramium* extract, yet the two former completed amylolysis in 6 days, while the latter required 9 days under the same conditions, as is shown in table V.

As a result of recent experiments, ACHALME and BRESSON[10] maintain that diffusion is an important factor in the rate of enzymatic action. They found that an increase in viscosity of the solution meant a like decrease in the rate of action of invertase upon saccharose. They also maintain that this is true for emulsin, trypsin, and for organic oxidases. As has been said, the viscosity of the algal extracts would about equal a 75 per cent solution of glycerine in water. That this condition influenced the amylolytic action of the extracts is not evident. It is true that the viscosity of the algal extracts greatly exceeded that of the commercial diastase and that action was very much more rapid in the latter than in the former; but this may have been due to some other factor, for although the extract from *Polysiphonia* was quite noticeably more viscous than that from *Ceramium*, yet the former completed digestion in two-thirds of the time required by the latter. Here, as before, we cannot draw definite conclusion, for there may have been a marked variation in purity between the two extracts. In some of the higher plants we find diastases that work very slowly, and it is not impossible that the algal diastases are also of this nature. It is a well known fact that by the addition of very small amounts of such substances as free mineral acids, neutral phosphoric acid compounds, salts of aluminium, and asparagin salts, the amylolytic action of the diastase from higher plants is very much accelerated, and it might be mentioned here that small amounts of aluminium acetate and sodium chloride had the same accelerating effect upon the algal extracts.

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[9] BELZUNG, E., Recherches morphologiques et physiologiques sur l'amidon et les grains de chlorophylle. Ann. Sci. Nat. Bot. VII. **5**:223–228. 1887.

[10] ALCHALME, P., and BRESSON, M., Influence de la viscosité du milieu sur les actions-diastasiques. Compt. Rend. **152**:1328–1330. 1911; also Du rôle de la viscosité dans les variations de l'action de l'invertine suivant les concentrations en saccharose. Compt. Rend. **152**:1420–1422. 1911.

The Van Tieghem tests were of interest not only because they corroborated the macrochemical tests, but also because they showed that the manner of attack of the algal extracts was such that the starch grains were corroded. This would indicate that at least the most active enzyme present was a translocation rather than a secretion diastase.

The contrast between the colors of the precipitates of the different mediums tested with Fehling's solution was very marked. The precipitates in the tubes containing the paste treated with algal extract was of the usual "brick-red" color. Paste treated with commercial diastase gave a light yellow precipitate, even though the tubes were allowed to stand for a considerable length of time before the test was made. This would indicate the presence of some colloid which prevented the deposit of free copper oxide. The precipitate in the tubes containing the algal extract showed that whether or not the viscous nature of the extract retarded amylolytic action, there were no colloids or other substances present which prevented a free deposit of the copper oxide.

Conclusion and summary

1. There is present in the red algae a diastase which will digest the starch of higher plants.
2. The manner of action of this enzyme indicates that it is at least partially composed of a translocation diastase.
3. The diastase of the red algae, like that of the higher plants, is probably not composed of a single enzyme, but of a series of amylases and dextrinases.
4. Judging by the action of the algal extract upon corn starch, the diastase is a rather slow-working enzyme.
5. The series of digestion processes resulting from the application of the algal diastase to corn starch would indicate that the substance composing the grains of the red algae is very similar to that of the starch grains of higher plants.

These experiments were begun at Woods Hole, Massachusetts, and completed at the University of Wisconsin. The writer wishes to take this opportunity to extend his thanks to Professor B. M. Duggar, at whose suggestion and under whose direction the work was begun, and also to Professor R. A. Harper and Professor W. G. Marquette for valuable assistance and helpful suggestion.

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